Amendments to the Specification

Please replace paragraph [0010.1] of the specification with the following paragraph:

[0010.1] What is required for the commercial viability of protein-based polymers is a cost of production that would begin to rival that of petroleum-based polymers. The potential to do so resides in low cost bioproduction. We have recently ~~deomonstrated~~ demonstrated a dramatic hyperexpression of an elastin protein-based polymer, (Gly-Val-Gly-Val-Pro (SEQ ID NO. 2))$_n$ or poly(~~GVGVP~~ Gly-Val-Gly-Val-Pro) (SEQ ID NO:2), which is a parent polymer for a diverse set of polymers that exhibit inverse temperature transitions of ~~hydorphobic~~ hydrophobic folding, and assembly as the temperature is raised through a transition range and which can exist in hydrogel, elastic and plastic states. Electron micrographs revealed formation of inclusion bodies in *E. coli* cells occupying up to 80-90% of the cell volume under optimal growth conditions (3a). The beauty of this approach is the lack of any need for extraneous sequences for the purposes of purification (4) or adequate expression. The usual strategy for expression of a foreign protein or ~~portein~~ protein-basedpolymer in an organism such as *E. coli* anticipates that the foreign protein will be injurious to the organism. Accordingly, the transformed cells are grown up to an appropriate stage before expression of the foreign protein is begun and expression is generally considered viable for only a few hours. The situation is quite different for the elastic protein-based polymer considered here. This may result in part due to the extraordinary biocompatibility exhibited by ~~(GVGVP)$_n$~~ (Gly-Val-Gly-Val-Pro, (SEQ ID NO:2))$_n$ and its related polymers. The elastic protein-based polymer, ~~(GVGVP)$_n$~~ (Gly-Val-Gly-Val-Pro, (SEQ ID NO:2))$_n$ and its γ-irradiation crosslinked matrix as well as related polymers and matrices appear to be ignored by a

range of animal cells and by tissues of the whole animal (5-7). ~~This chapter describes in detail methodologies to accomplish hyperexpression of a protein based polymer in *E. coli*.~~

Please replace paragraph [0010.2] of the specification with the following paragraph:

[0010.2] Construction of a synthetic protein-based polymer gene: As an illustration of an uninduced hyper-expression of a protein-based polymer in *E. Coli*, we have chosen a gene encoding 121 repeats of the elastomeric pentapeptide-gly-val-gly-val-pro (SEQ ID NO:2). This gene, $(gly\text{-}val\text{-}gly\text{-}val\text{-}pro)_{121}$, was constructed by ligase concatenation of DNA sequence encoding $(gly\text{-}val\text{-}gly\text{-}val\text{-}pro)_{10}$ and isolation of a concatener having 12 repeats of this monomer gene plus an additional C-terminal (gly-val-gly-val-pro) sequence encoded by a 3' cloning adaptor. The gene encoding $(gly\text{-}val\text{-}gly\text{-}val\text{-}pro)_{10}$ was synthesized and cloned into a multipurpose cloning plasmid from which it was then excised by digestion at flanking sites with the restriction endonuclease Pf1M1. A substantial amount of the Pf1M1 gene fragment was purified and self-ligated in the presence of limited amounts of synthetic double-stranded oligonucleotide adapters that provided the additional restriction sites needed for cloning the resulting concatemers. Pf1M1 cleaves at its recognition site in the DNA to leave two single-stranded extensions that are not self-complementary (i.e., nonpalindromic) but are only complementary to each other; therefore proper translational polaritity is maintained by head-to-tail tandem coupling of the monomer gene unites by ligase during the concatenation reaction.

Pro gly val gly val pro ~~(GVGVP)8~~ (Gly-Val-Gly-Val-Pro)8 gly val gly val pro gly val (SEQ ID NO. 16)
cgggatCCA GGC GTT GGT (SEQ ID NO. 17)----------CCA GGT GTT Ggatccg (SEQ ID NO. 22)
BamH1 Pf1M1 Pf1M1 BamH1

Above is the amino acid sequence and flanking restriction endonuclease sites of the basic polymer building block coding for (gly-val-gly-val-pro, (SEQ ID NO:3))$_{10}$. Using synthetic oligonucleotides and PCR, (gly-val-gly-val-pro, (SEQ ID NO:3))$_{10}$ was amplified with flanking BamH1 and Pf1M1 ends and the 121-mer gene was inserted into a pUC118 as a BamH1 fragment. For expression under control of the T7 polymerase gene promoter, a 121-mer gene was created by concatenation of the Pf1M1 10-mer fragment with terminal cloning adaptors and subsequently inserted into the expression vector pET-11d.

Please replace paragraph [0010.3] of the specification with the following paragraph:

[0010.3] Genes for the tricosapeptides ~~GVGVP~~ gly-val-gly-val-pro (SEQ ID NO. 2) ~~GVGFP~~ gly-val-gly-phe-pro (SEQ ID NO. 6) ~~GEGFP~~ gly-glu-gly-phe-pro (SEQ ID NO. 7) ~~GVGVP~~ gly-val-gly-val-pro (SEQ ID NO. 2) ~~GVGFP~~ gly-val-gly-phe-pro (SEQ ID NO. 6) ~~GFGFP~~ gly-phe-gly-phe-pro and ~~GVGVP~~ gly-val-gly-val-pro (SEQ ID NO. 14) ~~GVGFP~~ gly-val-gly-phe-pro (SEQ ID NO. 6) ~~GDGFP~~ gly-asp-gly-phe-pro (SEQ ID NO. 8) ~~GVGVP~~ gly-val-gly-val-pro (SEQ ID NO. 2) ~~GVGFP~~ gly-val-gly-phe-pro (SEQ ID NO. 6) ~~GFGFP~~ gly-phe-gly-phe-pro, analogous to compounds LXII and LIX, respectively, were constructed using synthetic oligonucleotides. The double-stranded DNA sequence of these genes with the corresponding amino acid sequence is the following (**Equation 1**):

BamH-1 Pf1M-1

5' – GAGGATCCAGGCGTTGGGGTACCGGGTGTTGGCTTCCCG (SEQ ID NO. 9)

3' – ctcctaggtccgcaaccccatggcccacaaccgaagggc (SEQ ID NO. 10)

~~G V G V P G V G F P~~ Gly val gly val pro gly val gly phe pro (SEQ ID NO. 11)

GGTGAMGGTTTCCCGGGCGTTGGTGTGccg (SEQ ID NO. 12)

ccactkcCAAAGGGCCCGGCAACCACACGGC (SEQ ID NO. 13)

~~G X G F P G V G V P~~ Gly X gly phe pro gly val gly val pro (SEQ ID NO. 14)

Pf1M-1 BamH-1

ggtgtaggctttcgggtttcggattcccaggcgttggatccag – 3' (SEQ ID NO. 15)

CCACATCCGAAAGGCCCAAAGCCTAAGGGTCCGCAACCTAGGTC –5'(SEQ ID NO. 18)

~~G V G F P G F G F P~~ gly val gly phe pro gly phe gly phe pro (SEQ ID NO. 19)

Where nucleotides M/k = A/t when amino acide X = E and M/k = C/g when X = D. For each gene, two single-stranded oligonucleotides, indicated by the upper-case letters in the sequence, where annealed through their overlapping regions of complementarily(dashed line) and extended from their 3' ends with DNA polymerase and deoxynucleotide-triphosphates to give the full-length, double-stranded molecule.

Please replace paragraph [0010.4] of the specification with the following paragraph:

[0010.4] ~~Amino acid sequence and flanking restriction endonuclease sites of the basic polymer building block coding for (GVGVP gly-val-gly-val-pro)$_{10}$. Using synthetic oligonucleotides and PCR, (GVGVP gly-val-gly-val-pro)$_{10}$ was amplified with flanking BamH1 and Pf1M1 ends and the 121-mer gene was inserted into a pUC118 as a BamH1 fragment. For expression under control of the T7 polymerase gene promoter, a 121-mer gene was created by concatenation of the Pf1M1 10-mer fragment with terminal cloning adaptors and subsequently inserted into the expression vector pET-11d.~~ The initial gene which encodes 10 repeating unites of the elastomeric pentapeptide gly-val-gly-val-pro (SEQ ID NO. 2) , i.e. (gly-val-gly-val-pro)$_{10}$

(SEQ ID NO. 3) was constructed by using polymerase chain reaction (McPherson et al. 1992). Higher molecular weight polymer genes were then made by concatenation / ligation reaction using suitable adaptor oligonucleotide fragments. Details of a series of these gene constructs have been published elsewhere (McPherson et al. 1996). These higher molecular weight polymer genes were subsequently cloned into pUC118 as a BamH1-BamH1 fragment.

Please add the following new paragraphs to the specification:

[0010.4.1] ~~GVGVP Gly-val-gly-val-pro. This gene, (GVGVP gly-val-gly-val-pro)$_{121}$, was constructed by ligase concatenation of DNA sequence encoding (GVGVP gly-val-gly-val-pro)$_{10}$ and isolation of a concatener having 12 repeats of this monomer gene plus an additional C-terminal (GVGVP gly-val-gly-val-pro) sequence encoded by a 3' cloning adaptor (*10a*). The gene encoding (GVGVP gly-val-gly-val-pro)$_{10}$ was synthesized and cloned into a mulitpurpose multipurpose cloning plasmid from which it was then excised by digestion at flanking sites witht herestriction endonuclease Pf1M1 (Fig. 1). A substantial amount of the Pf1M1 gene fragment was purified and self-ligated in the presence of limited amounts of synthetic double-stranded oligonucleotide adapters that provided the additional restriction sites needed for cloning the resulting concatemers. Pf1M1 cleaves at its recognition site in the DNA to leave two single-stranded extensions that are not self-complementary (i.e., nonpalindromic) but are only complementary to each other; therefore proper translational polaritity is maintained by head-to-tail tandem coupling of the monomer gene unites by ligase during the concatenation reaction.~~ Synthetic Oligonucleotides. The universal sequencing primer was obtained from New England Biolabs. All other oligonucleotides either were synthesized on an Applied Biosystems

automated DNA synthesizer by the University of Alabama at Birmingham Cancer Center DNA Synthesis Core Facility or were purchased from Oligos, Etc.

[0010.4.2] Construction of Synthetic Gene. A DNA sequence coding for (VPGVG SEQ ID NO. 1)$_{10}$ was constructed using two synthetic oligonucleotides, each 85 bases in length, with 3'-overlapping complementary ends. They had the following sequences:

5'-GTTCCGGGTGTTGGTGTACCGGGTGTTGGTGTGCCGGGTGTTGGTGTTCCGGGC GTAGGCGTACCGGGCGTAGGCGTGCCGGGCG-3' (SEQ ID NO. 20)

5'-ACCTACACCCGGAACGCCCACACCCGGCACGCCCACGCCCGGTACGCCCACGCC CGGAACGCCTACGCCCGGCACGCCTACGCCC-3' (SEQ ID NO. 21)

Briefly, the 3' ends were annealed through a 20-base region of complementarity and extended with AMV reverse transcriptase and deoxynucleotides to provide complementary strands of 150 bases.

[0010.4.3] Polymerase Chain Reaction (PCR). PCR (Saiki et al., 1987) reactions were performed in a total volume of 100μL containing approximately 1ng of plasmid DNA as template and 100pmol of each primer in a mixture of 10mM Tris-HCl, pH 8.3, 50mM KCl, 1.5mM $MgCl_2$, 200 mM each deoxynucleotidetriphosphate, and 2.5 unites of recombinant *Thermus aquaticus* DNA polymerase (Amplitaq, Perkin-Elmer Cetus). The above mix was overlaid with an equal volume of mineral oil (reagent-grade, Sigma) and subjected to 30 cycles of 94°C for 1 min, 52°C for 3 min, and 72°C for 3 min in a Perkin-Elmer Cetus DNA thermal cycler, with minimal ramp time between steps. In each case, a DNA fragment of the desired size was purified by first digesting the PCR product with the appropriate restriction enzymes, followed by electrophoresis through 6% acrylamide, band excision, electroelution into dialysis tubing, and precipitation with ethanol.

Please replace paragraph [0018] of the specification with the following paragraph:

[0018] Alternatively, the Agrobacterium-mediated method is manageable in the university setting and has been used successfully to introduce 2,4-D resistance into cotton (Bayley et al., 1992). However, a disadvantage of this technique is that the subsequent regeneration is not cultivar-independent (Trolinder and Goodin, 1987, 1988). Consequently, desirable traits in the transformed plants must be subsequently crossed into current production varieties, such as *Gossypium hirsutum* L. var Coker 312 and 5110, T25, Y169, *Paymaster* 303, *Paymaster* 784 and RQSX-1-1, the *G. hirsutum* x *G. barbadense* hybrid. After completion of recombinant DNA vector constructions, cotton transformation will be carried out in Dr. Haigler's laboratory (Texas Tech, Lubbock, TX). Fiber qualities of genetically engineered cotton will be analyzed at Auburn University and in collaboration with Dr. Rajasckaran, (USDA Southern Regional Laboratories, New Orleans, LA).